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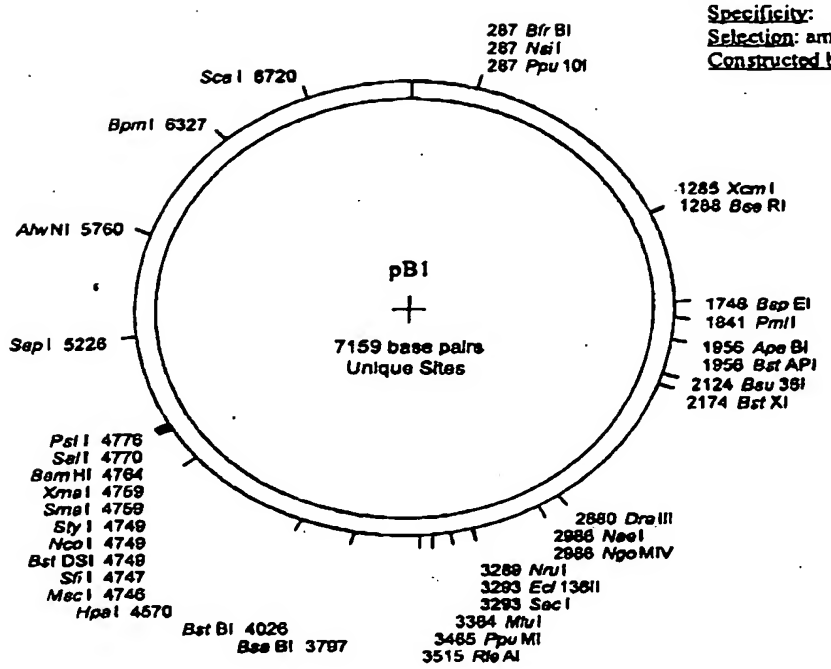
pB1

Alias: pAS2DD
Application: 2HY (bait)
Backbone:
Specifisity:
Selection: ampicillin
Constructed by:



Oligo 160

gagagtagtaacaaaggtc AAAGACAGTTGACTGTATCGCCG GAA TTT AT

Sfi I **Sma I** **BamH I** **Sal I** **Pst I**

G GCC ATG GAG GCC CCG GGG ATC CGT CGA CCT GCA GCC

Nco I

Oligo 161

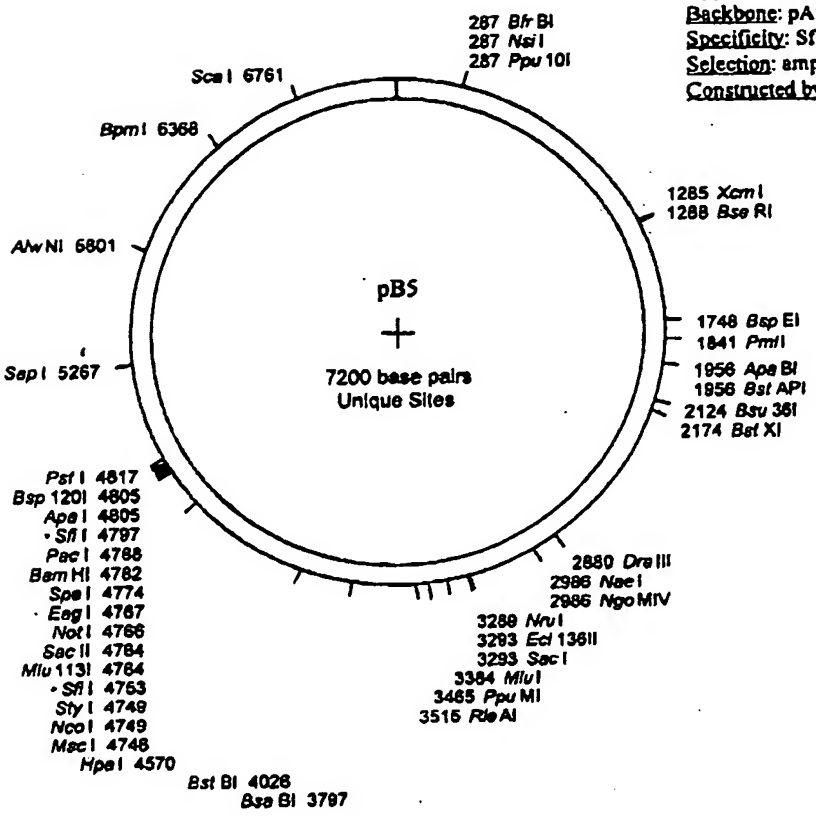
AAG CTA ATT ccgggcgaatttcttatg

Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'
Oligo 161 5' CATAAGAAATTCGCCCGG 3'

FIGURE 1

pB5

Alias: pAS2DDNS1
Application: 2HY (bait)
Backbone: pAS2DD
Specificity: Sfi non-oriented
Selection: ampicillin
Constructed by: SW



Oligo 160

gagagtagtaacaaaggtc AAAGACAGTTGACTGTATCGCCG GAA TTT ATG

Sfi I **Sac II** **Spe I** **Bam HI**

GCC ATG GCC GCA GGG GCC GCG GCC GCA CTA GTG GGG ATC C

Nco I **Not I**

STOP **Sfi I** **Pst I**

TT AAT TAA GGG CCA CTG GGG CCC CTC GAC CTG CAG CCA

Pac I

Oligo 161

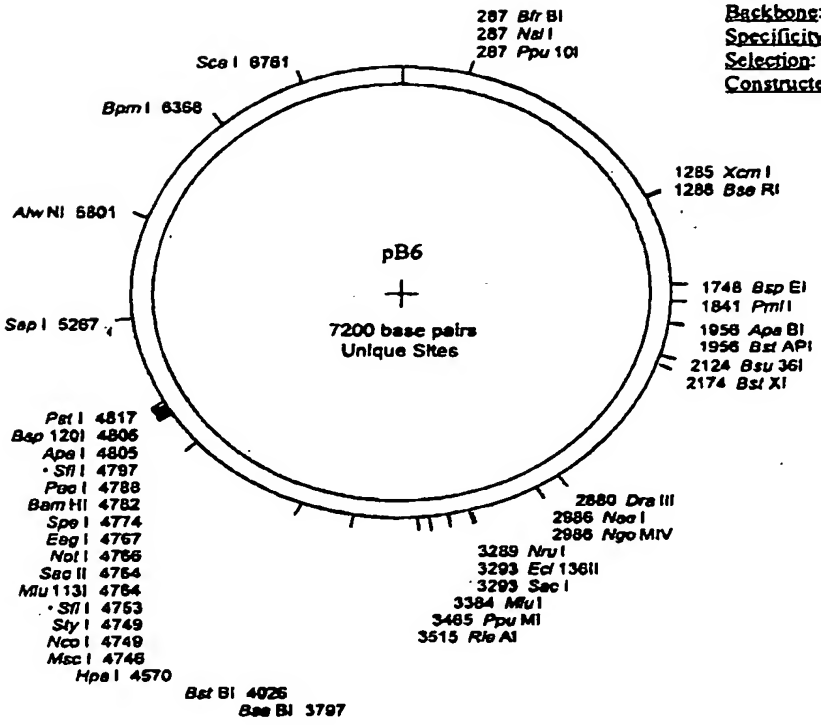
AGC TAA TT ccgggcgaatttcttatg

Olig 160 5' GAGAGTAGTAACAAAGGTC 3'
Oligo 161 5' CATAAGAAATTCGCCCGG 3'

FIGURE 2

pB6

Application: 2HY (bait)
Backbone: pAS2DD
Specificity: Sfi oriented
Selection: ampicillin
Constructed by: SW



Oligo 160

gagagtagtaacaaaggtcAAAGACAGTTGACTGTATCGCCG GAA TTT ATG

Sfi I — Sac II — Spe I — Bam HI

GCC ATG GCC GGA CGG GCC GCG GCC GCA CTA GTG GGG ATC C

Nco I — Not I

STOP — Sfi I — Apa I — Pst I

TT AAT TAA GGG CCA CTG GGG CCC CTC GAC CTG CAG CCA

Pac I

Oligo 161

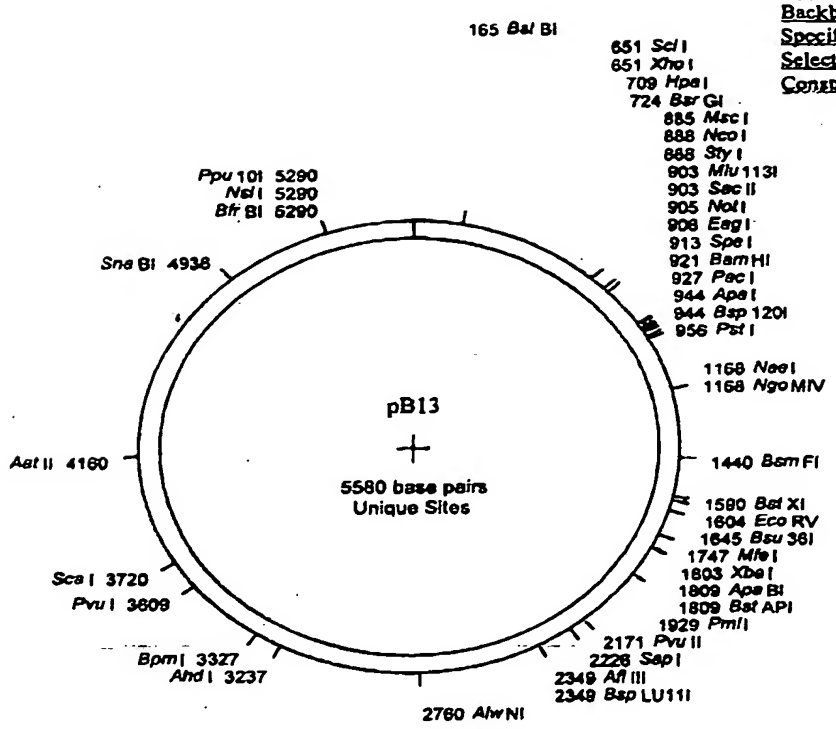
AGC TAA TTccgggcgaatttcttatg

Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'
Oligo 161 5' CATAAGAAATTCGCCCGG 3'

FIGURE 3

pB13

Alias: pGBT9NS1
Application: 2HY (bait)
Backbone: pGBT9
Specificity: Sfi non-oriented
Selection: ampicillin
Constructed by: CR



Oligo 160

gagagtagtaacaaaggtc AAAGACAGTTGACTGTATCGCCG GAA TTT ATG

Sfi I **Sac II** **Spe I** **Bam HI**

GCC ATG GCC GCA GGG GCC GCG GCC GCA CTA GTG GGG ATC C

Nco I **Not I**

STOP **Sfi I** **Pst I**

TT AAT TAA GGG CCA CTG GGG CCC CTC GAC CTG CAG CCA

Pac I

Oligo 161

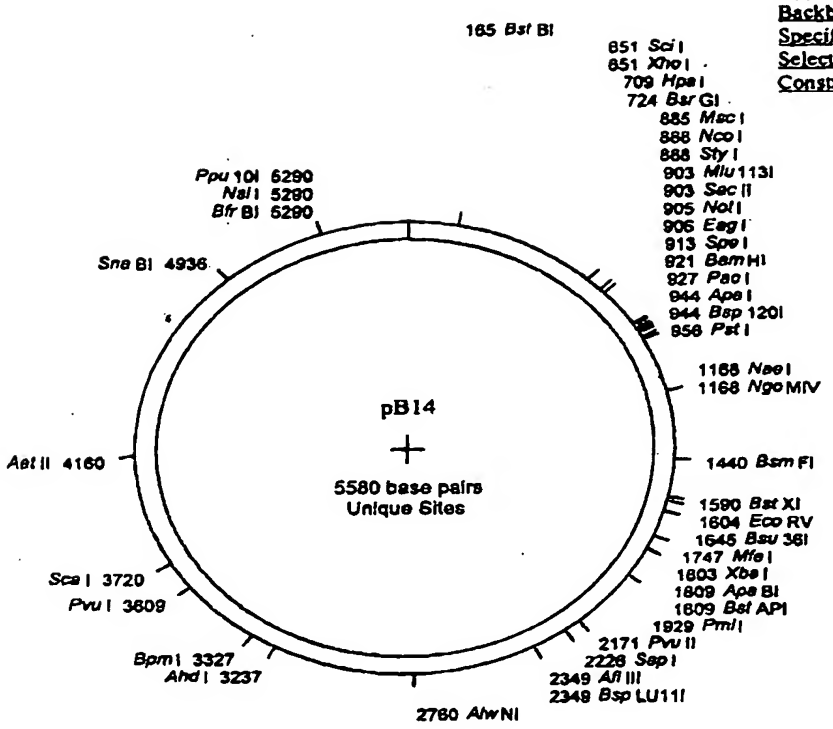
AGC TAA TT ccgggcgaatttcttatg

Oligo 160 5' GAGAGTAGTAACAAAGGTC 3'
Oligo 161 5' CATAAGAAATTCGCCCGG 3'

FIGURE 4

pB14

Alias: pGBT9NS2
Application: 2HY (bait)
Backbone: pGBT9
Specificity: Sfi oriented
Selection: ampicillin
Constructed by: CR



Oligo 160

gagagtagtaacaaaggtc AAAGACAGTTGACTGTATCGCCG GAA TTT ATG

Sfi I — Sac II — Spe I — Bam HI

GCC ATG GCC GGA CGG GCC GCG GCC GCA CTA GTG GGG ATC C

Nco I — Not I

STOP — Sfi I — Apa I — Pst I

TT AAT TAA GGG CCA CTG GGG CCC CTC GAC CTG CAG CCA

Pac I

Oligo 161

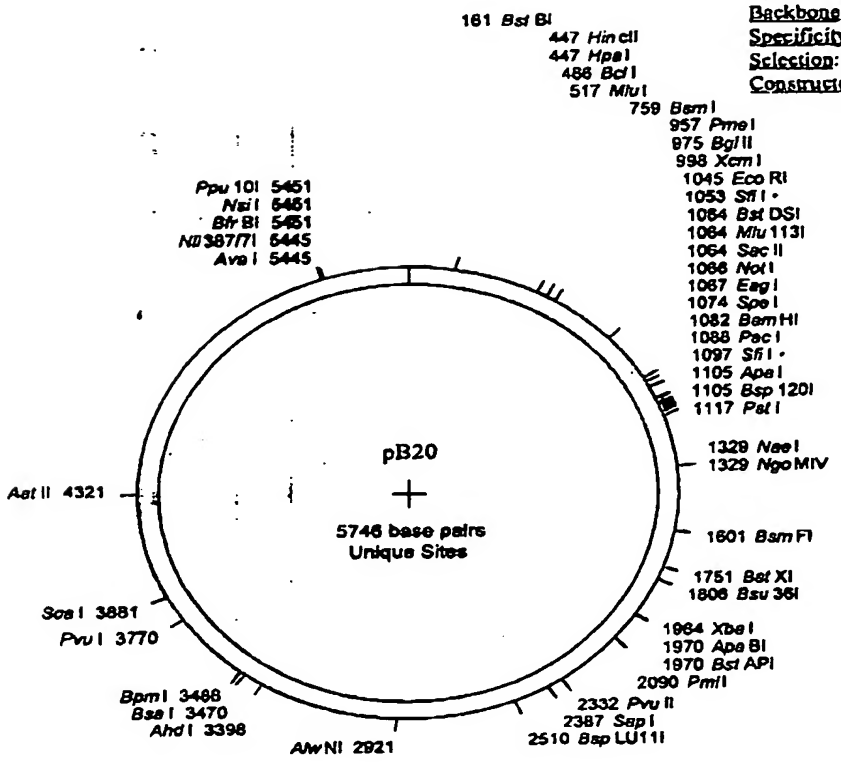
AGC TAA TT ccgggcgaatttcttatg

Oligo 160 5' **GAGAGTAGTAACAAAGGTC** 3'
Oligo 161 5' **CATAAGAAATTCGCCCGG** 3'

FIGURE 5

pB20

Alias: pLex10NS2
Application: 2HY (bait)
Backbone: pLex10 (pB9)
Specificity: Sfi-oriented
Selection: ampicillin
Constructed by: LD



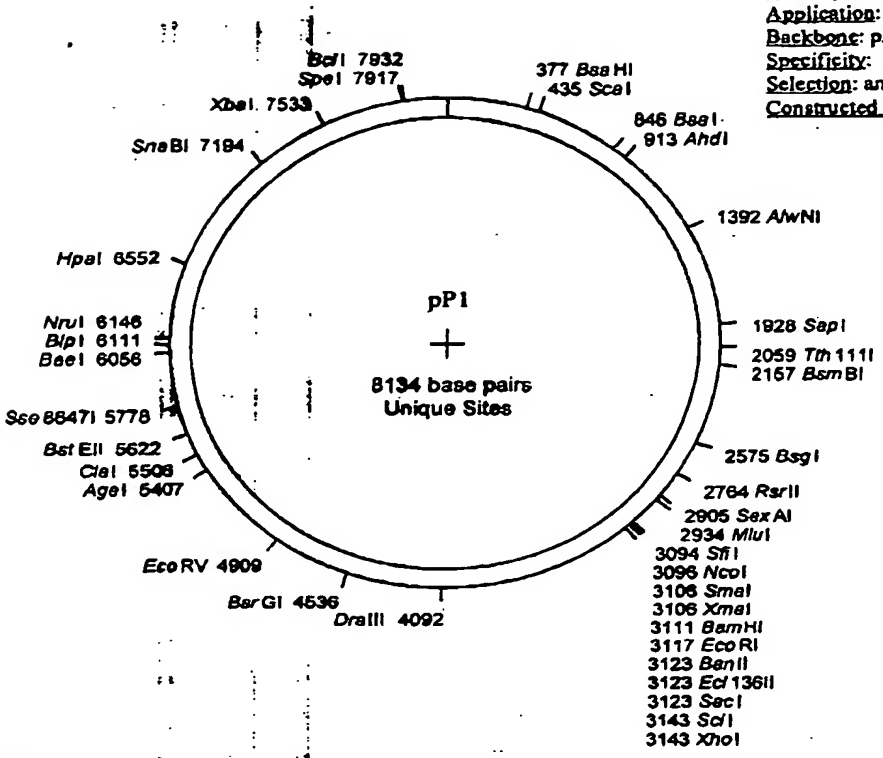
EcoR I	Sfi I		Not I	Spe I	BamH I
GAA TTC	GGG GCC GGA CGG	GCC GCG GCC	GCA CTA	GTG	GGG ATC C
		Sac II			

STOP					
TT AAT	TAA	GGG CCA CTG GGG CCC CTC GAC		CTG	CAG
Pac I		Sfi I		Pst I	

FIGURE 6

pP1

Alias : pACTIIst
Application: 2HY (prcy)
Backbone: pACTII
Specificity:
Selection: ampicillin
Constructed by:



ABS1

cgtttggaatcactacagg GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90 Bgl II

cgatgatgaagataccccaccaaa CCCAAAAAAAAGAGATCTGTATGGCTTACCCATACGATGTTCCAG

Sfi I Sma I BamH I

ATTACGCTAGCTTGGGTGGTCATATGGCC ATG GAG GCC CCG GGG ATC CGA ATT

Nco I

Sac I Xho I Bgl II

CGA GCT CGA CTA GCT AGC TGA CTC GAG AGA TCT ATGAAT

cgtagatactgaaaaacccc GCAAGTT cacttcaactgtgcatcgtg caccatctcaatttc

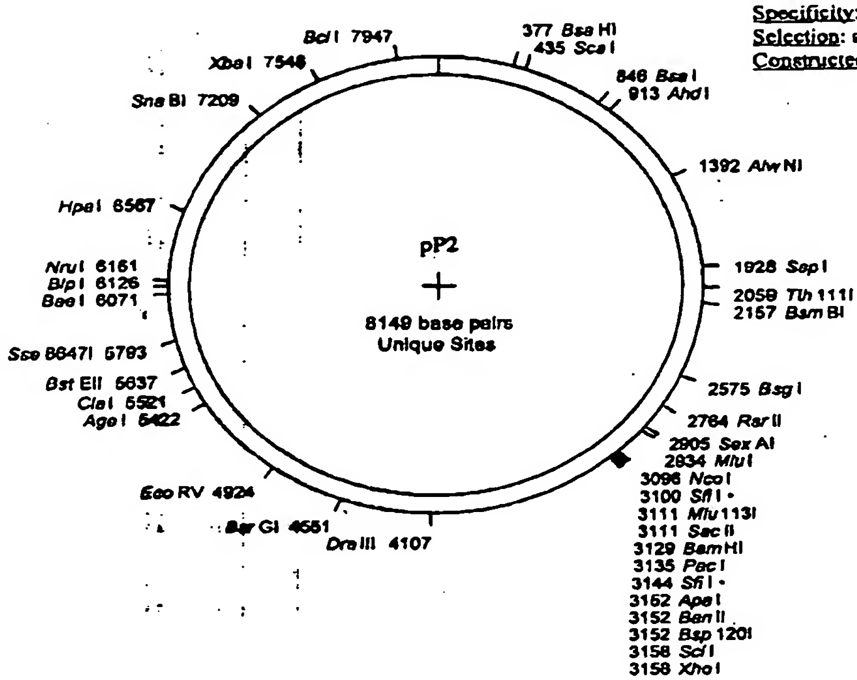
162 ABS2 53

ABS1 5' CGTTTGGAATCACTACAGG 3'
JC90 5' CGATGATGAAGATACCCCACCAAA 3'
162 5' GGGGTTTTTCAGTATCTACG 3'
ABS2 5' CACGATGCACAGTTGAAGTG 3'
53 5' GAAATTGAGATGGTGCACGATGCAC 3'

FIGURE 7

pP2[8]

<u>Application</u>: 2HY (prey)
<u>Backbone</u>: pACTIIst
<u>Specificity</u>:Sfi non-oriented
<u>Selection</u>: ampicillin
<u>Constructed by</u>:SW



ABS1

CG [cgtttggaatcactacagg] GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90 — Bgl II

[cgatgatgaagataccccaccaaa] CCCAAAAAAAGAGATCTGTATGGCTTACCCATACGATGTTCCAG

Sfi I — Sac II

ATTACGCTAGCTTGGGTGGTCATATGGCC ATG GCC GCA GGG GCC GCG GCC GCA

Nco I

BamH I — Pac I

CTA GTG GGG ATC CTT AAT [TAA] GGG CCA CTG GGG CCC CTC GAG AGA TCT

Stop

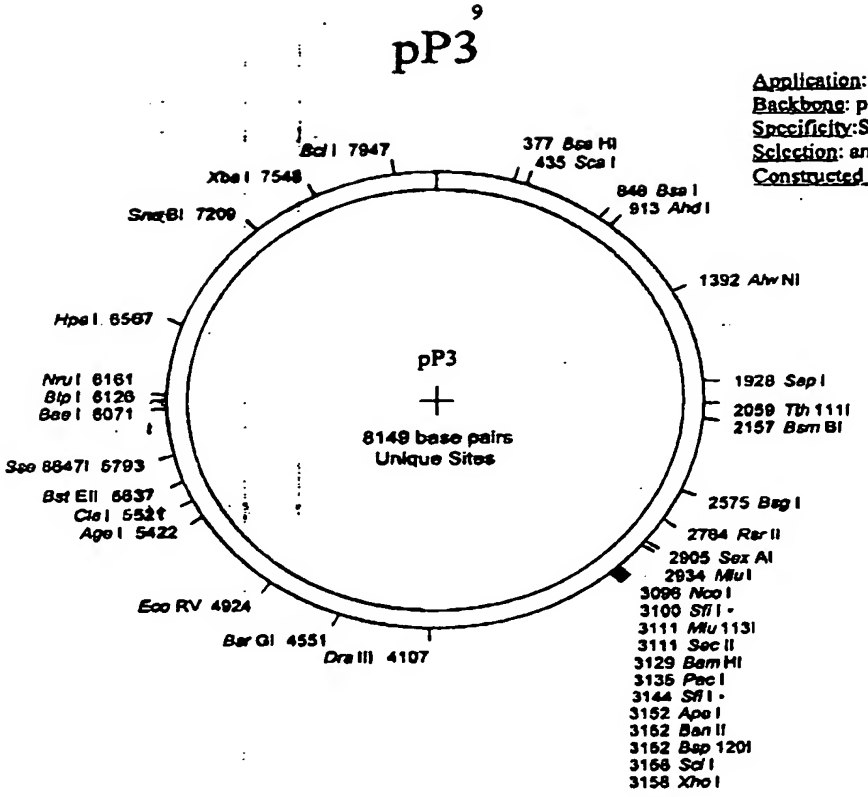
ATGAAT [cgtagatactgaaaaacccc] GCAAGTT [cacttcaactgtgcatcgtg] [caccatctcaatttc]

162 — ABS2 — 53

ABS1 5' CGTTTGGAATCACTACAGG 3'
JC90 5' CGATGATGAAGATACCCCACCAAA 3'
162 5' GGGGTTTTTCAGTATCTACG 3'
ABS2 5' CACGATGCACAGTTGAAGTG 3'
53 5' GAAATTGAGATGGTGCACGATGCAC 3'

FIGURE 8

pP3



ABS1

CG cgtttggaatcactacagg GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90 Bgl II

cgatgatgaagataccccaccaaa CCCAAAAAAAAGAGATCTGTATGGCTTACCCATACGATGTTCCAG

Sfi I Sac II

ATTACGCTAGCTTGGGTGGTCATATGGCC ATG GCC GGA CGG GCC GCG GCC GCA

Nco I

BamH I Pac I

CTA GTG GGG ATC CTT AAT TAA GGG CCA CTG GGG CCC CTC GAG AGA TCT

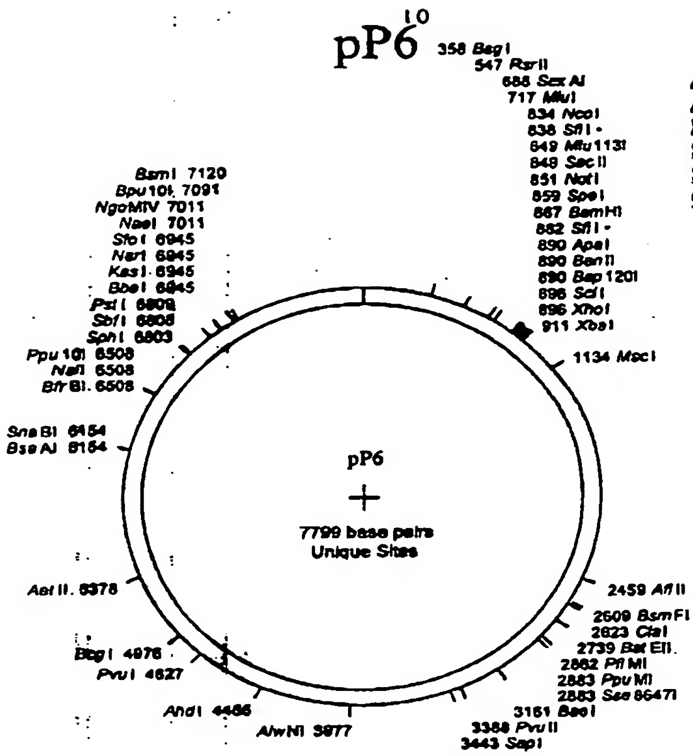
Stop

ATGAAT cgtagatactgaaaaacccc GCAAGTT cacttcaactgtgcatcgtg caccatctcaatttc

162 ABS2 53

ABS1 5' CGTTTGGAATCACTACAGG 3'
JC90 5' CGATGATGAAGATACCCCACCAAA 3'
162 5' GGGGTTTTTCAGTATCTACG 3'
ABS2 5' CACGATGCACAGTTGAAGTG 3'
53 5' GAAATTGAGATGGTGCACGATGCAC 3'

FIGURE 9


pP6
358 BsgI
547 RsrII
686 SexAI
717 MluI
834 NcoI
838 SfiI •
849 Mlu1131
848 SacII
851 NotI
859 SpeI
867 BamHI
882 SfiI •
890 ApaI
890 BanII
890 Bsp1201
896 SciI
896 XhoI
911 XbaI
1134 MscI
2459 AflII
2609 BsmFI
2823 ClaI
2739 BstEII
2862 PflMI
2883 PpuMI
2883 Sse8647I
3151 BsaI
3388 PvuII
3443 SapI
AlwNI 3977
AhdI 4466
PvuI 4827
BsgI 4976
AatII. 5378
BsaAI 6154
SnaBI 6154
BfrBI. 6508
NsiI 6508
Ppu10I 6508
SphI 6803
SbfI 6808
PstI 6808
BbeI 6945
KasI 6945
NarI 6945
SfoI 6945
NaeI 7011
NgoMIV 7011
Bpu10I 7091
BsmI 7120
pP6
7799 base pairs
Unique Sites

Alias: pGAD3S2XNS1
Application: 2HY (prey)
Backbone: pGAD3S2X
Specificity: Sfi non-oriented
Selection: ampicillin
Constructed by: SW

ABS1

[cgtttggaatcactacagg] GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90

[cgatgatgaagataccccaccaaa] CCCAAAAAAAGAGATCCTAGAACTA

Sfi I, Sac II, Spe I, Bam HI

GCC ATG GCC GCA GGG GCC GCG GCC GCA CTA GTG GGG ATC C

Nco I, Not I

STOP, Sfi I, Xho I, Xba I

TT AAT [TAA] GGG CCA CTG GGG CCC CTC GAG [TAG] C[TA G]TG T[CT AG]A

STOP STOP STOP

GGCCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCG TCGTTTTA

CAACGTCGTGACTGGGAAAACCCTGATCTATGAAT [cgtagatactgaaaaacccc] GCAA

162

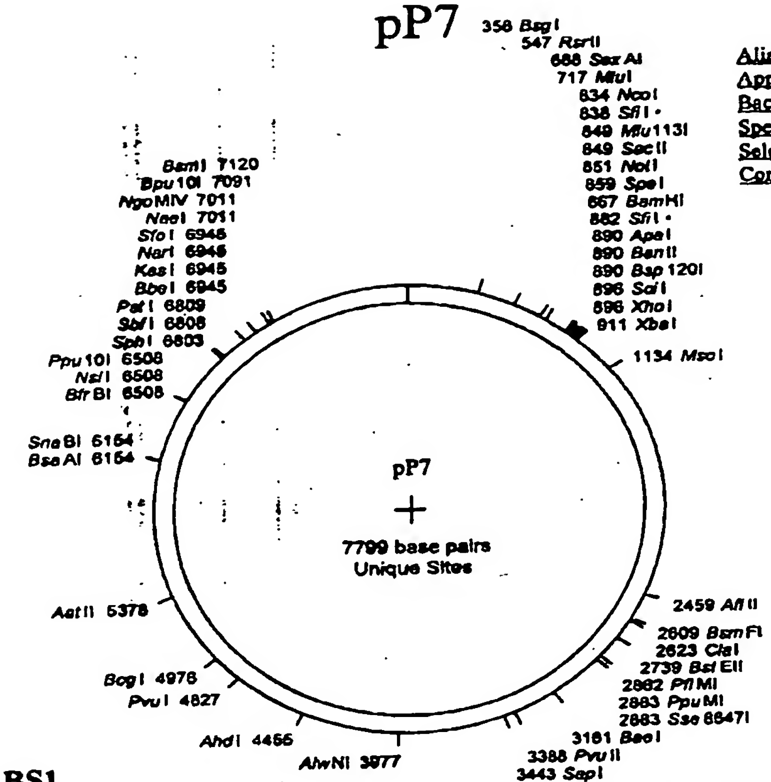
GTT [cacttcaac[tgtgcatcgtg] caccatctcaatttctttc]

ABS2 53

ABS1 5' CGTTTGGAATCACTACAGG 3'
JC90 5' CGATGATGAAGATACCCCACCAAA 3'
162 5' GGGGTTTTTCAGTATCTACG 3'
ABS2 5' CACGATGCACAGTTGAAGTG 3'
53 5' GAAATTGAGATGGTGCACGATGCAC 3'

FIGURE 10

pP7



358 BsgI
547 RsrII
688 SexAI
717 MluI
834 NcoI
838 SfiI •
849 Mlu113I
849 SacII
851 NotI
859 SpeI
867 BamHI
882 SfiI •
890 ApaI
890 BanII
890 Bsp120I
896 SalI
896 XhoI
911 XbaI
1134 MscI
2459 AflII
2609 BsmFI
2623 ClaI
2739 BstEII
2882 PflMI
2883 PpuMI
2883 Sse8647I
3181 BsaI
3388 PvuII
3443 SapI
AlwNI 3977
AhdI 4466
PvuI 4827
BcgI 4976
AatII 5378
BsaAI 6154
SnaBI 6154
BfrBI 6508
NsiI 6508
Ppu10I 6508
SphI 6803
SbfI 6808
PstI 6809
BbeI 6945
KasI 6945
NarI 6945
SfoI 6945
NaeI 7011
NgoMIV 7011
Bpu10I 7091
BsmI 7120
pP7
7799 base pairs
Unique Sites

Alias: pGAD3S2XNS2
Application: 2HY (prey)
Backbone: pGAD3S2X
Specificity: Sfi oriented
Selection: ampicillin
Constructed by: SW

ABS1

[cgtttggaatcactacagg] GATGTTTAATACCACTACAATGGATGATGTATATAACTATCTATT

JC90

[cgatgatgaagataccccaccaaa] CCCAAAAAAAGAGATCCTAGAACTA

Sfi I, Sac II, Spe I, Bam HI

GCC ATG GCC GGA CGG GCC GCG GCC GCA CTA GTG GGG ATC C

Nco I, Not I

STOP, Sfi I, Xho I, Xba I

TT AAT [TAA] GGG CCA CTG GGG CCC CTC GAG [TAG] C[TA G]TG TC[T AG]A

STOP STOP STOP

GGCCCGGTACCCAATTCGCCCTATAGTGAGTCGTATTACAATTCACTGGCCGTCGTTTTA

CAACGTCGTGACTGGGAAAACCCTGATCTATGAAT [cgtagatactgaaaaacccc] GCAA

162

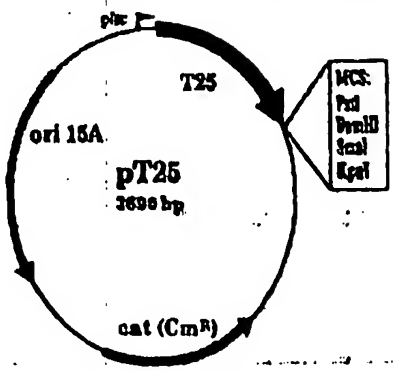
GTT [cacttcaac[gtgcatcgtg] [caccatctcaatttcttt]

ABS2 53

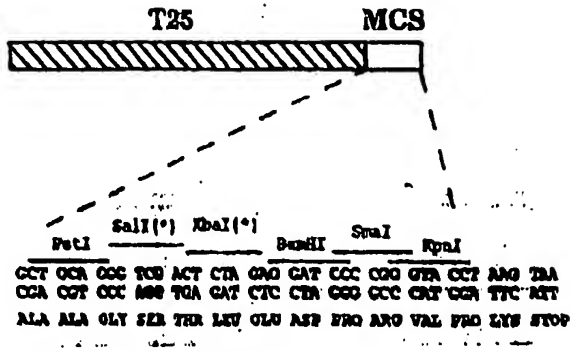
ABS1 5' CGTTTGGAATCACTACAGG 3'
JC90 5' CGATGATGAAGATACCCCACCAAA 3'
162 5' GGGGTTTTTCAGTATCTACG 3'
ABS2 5' CACGATGCACAGTTGAAGTG 3'
53 5' GAAATTGAGATGGTGCACGATGCAC 3'

FIGURE 11

VECTORS EXPRESSING THE T25 FRAGMENT

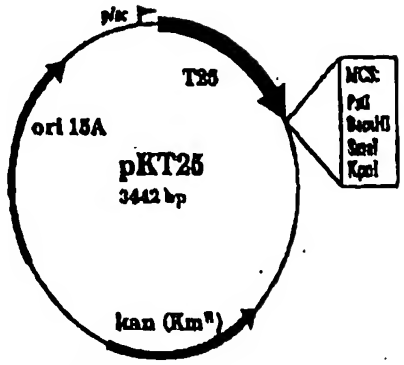


Derivative of pACYC184

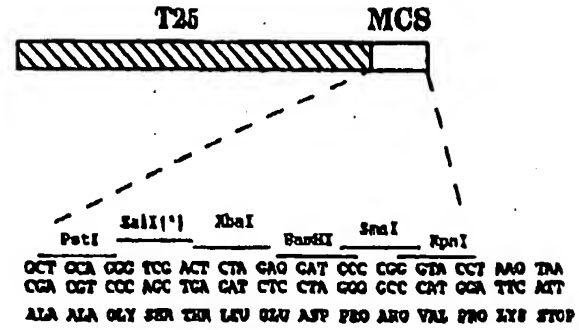


(*) Restriction sites are not unique

FIGURE 12



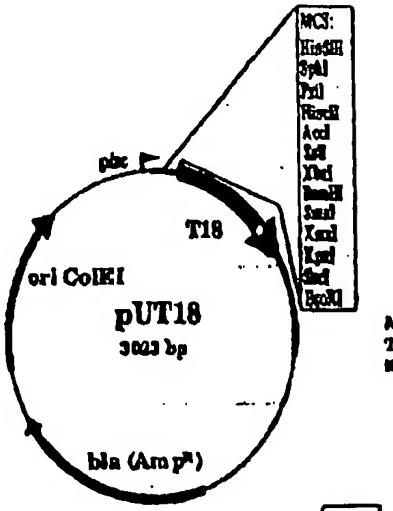
Derivative of pSU40

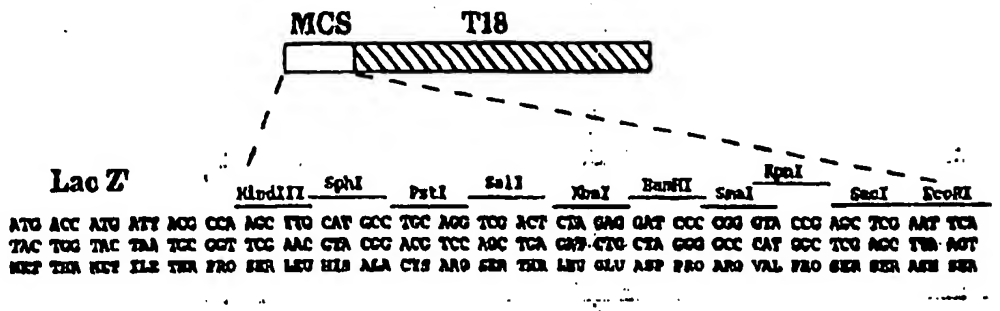


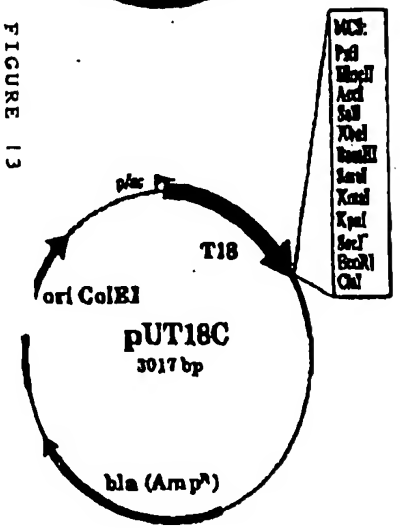
(*) Restriction site is not unique

FIGURE 12

VECTORS EXPRESSING THE T18 FRAGMENT







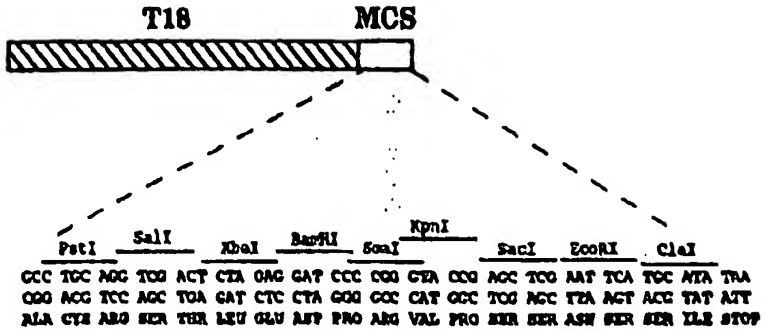


FIGURE 13

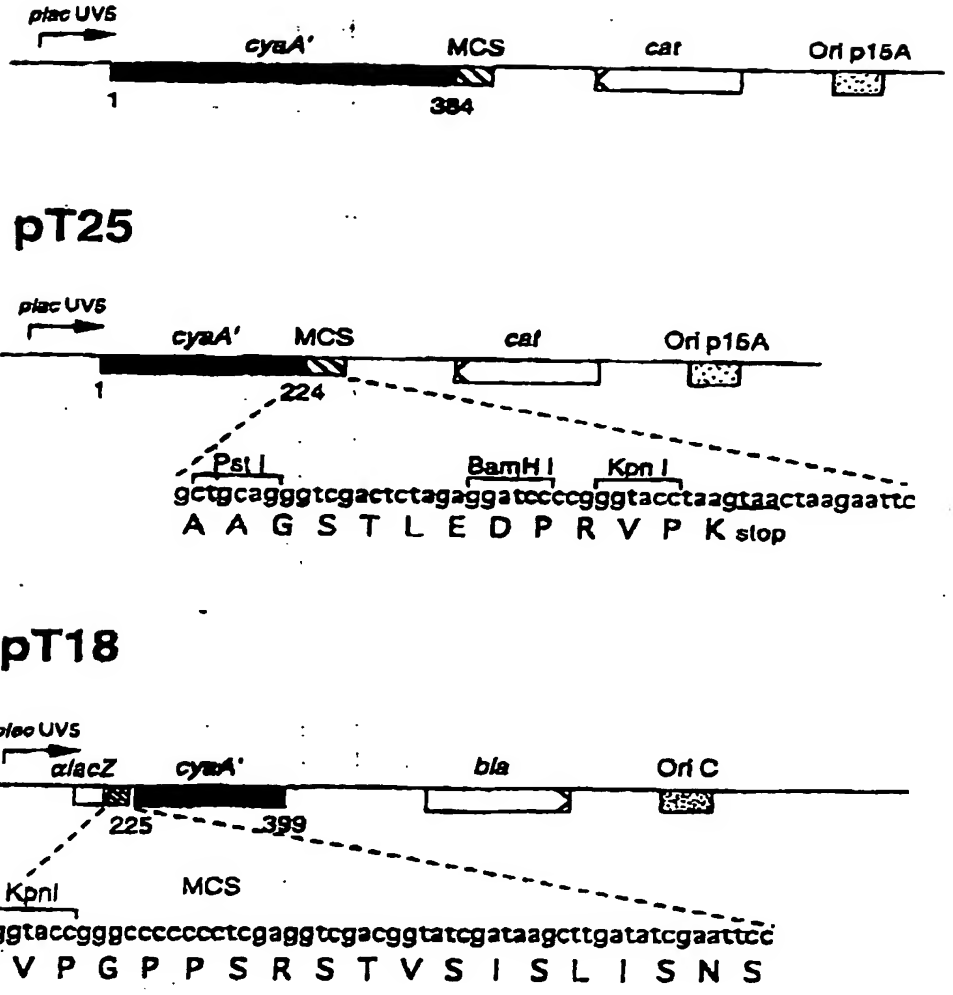

pCmAHL1
ptac UV5
cyaA'
MCS
cat
Ori p15A
1
384
pT25
ptac UV5
cyaA'
MCS
cat
Ori p15A
1
224
Pst I
BamH I
Kpn I
gctgcagggtcgactctagaggatccccgggtacctaagtaactaagaattc
A A G S T L E D P R V P K stop
pT18
ptac UV5
αlacZ
cyaA'
bla
Ori C
225
399
KpnI
MCS
tgggtaccgggcccccctcgaggtcgacggtatcgataagcttgatatcgaattcc
W V P G P P S R S T V S I S L I S N S

FIGURE 14

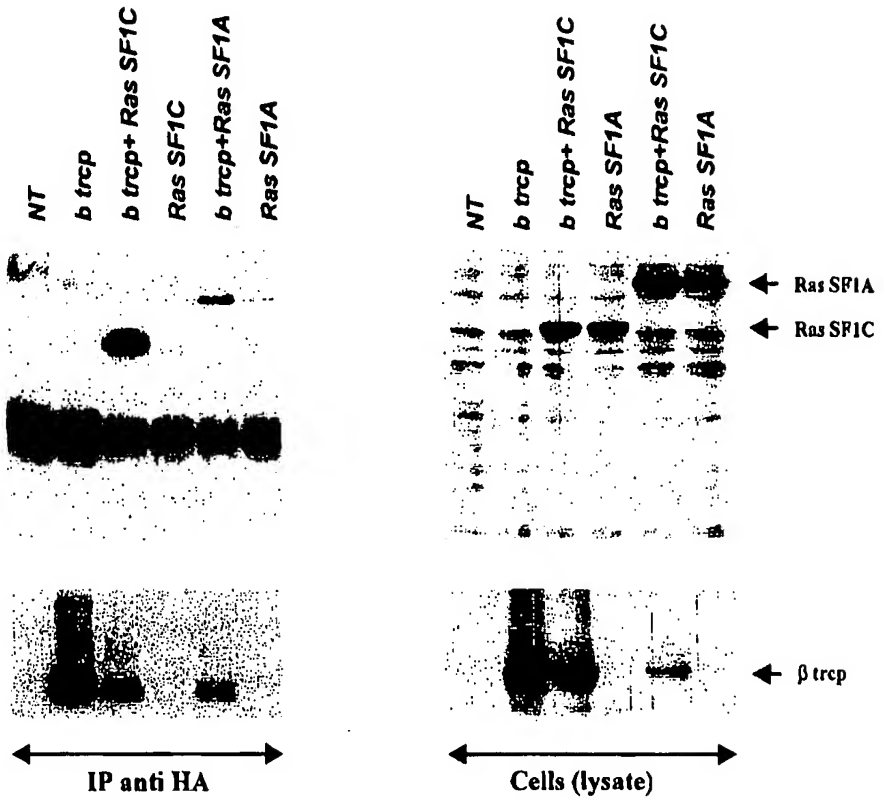

NT
b trcp
b trcp+ Ras SF1C
Ras SF1C
b trcp+Ras SF1A
Ras SF1A
NT
b trcp
b trcp+ Ras SF1C
Ras SF1A
b trcp+Ras SF1C
Ras SF1A
Ras SF1A
Ras SF1C
β trcp
IP anti HA
Cells (lysate)

Figure 15

Figure 16

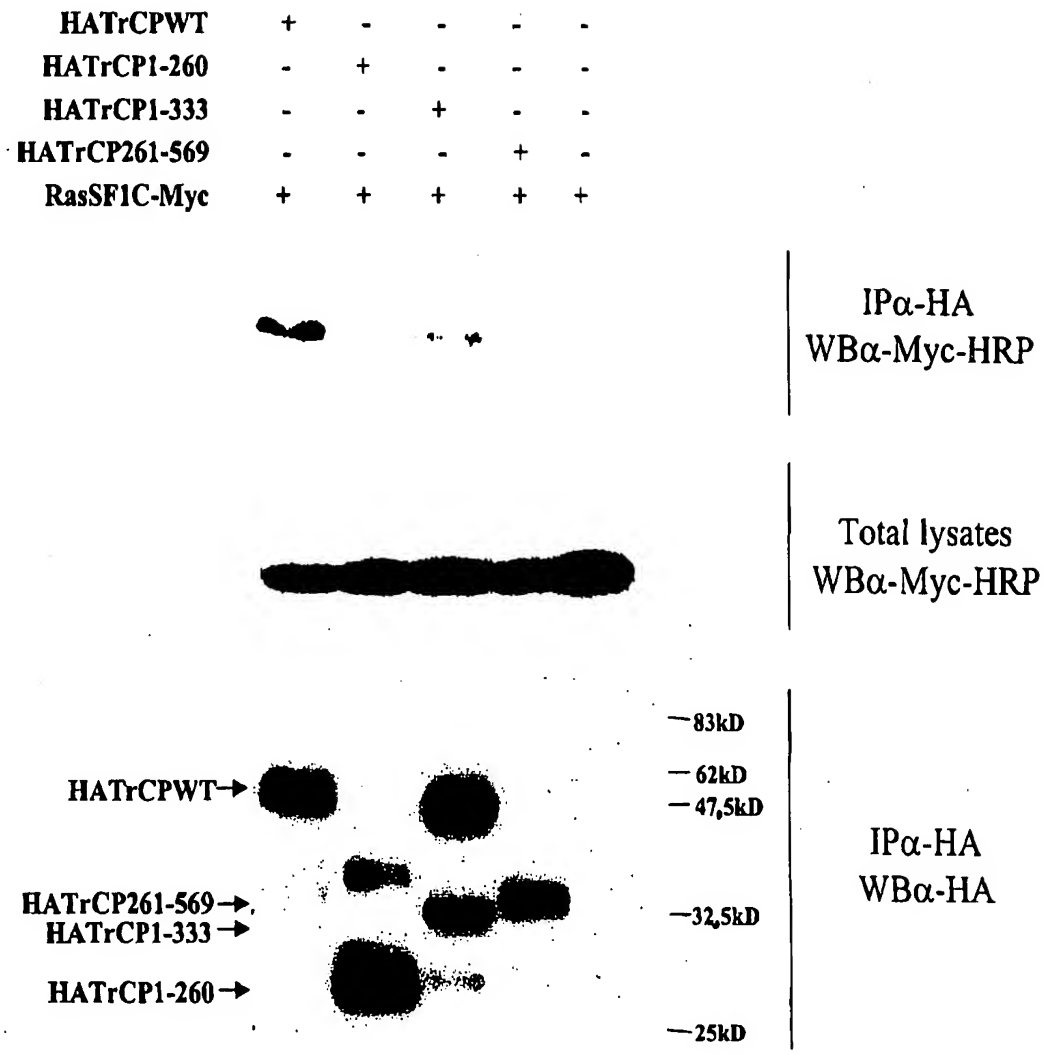

HATrCPWT + - - - -
HATrCP1-260 - + - - -
HATrCP1-333 - - + - -
HATrCP261-569 - - - + -
RasSF1C-Myc + + + + +
IPα-HA
WBα-Myc-HRP
Total lysates
WBα-Myc-HRP
83kD
62kD
47,5kD
32,5kD
25kD
HATrCPWT
HATrCP261-569
HATrCP1-333
HATrCP1-260
IPα-HA
WBα-HA

Figure 17

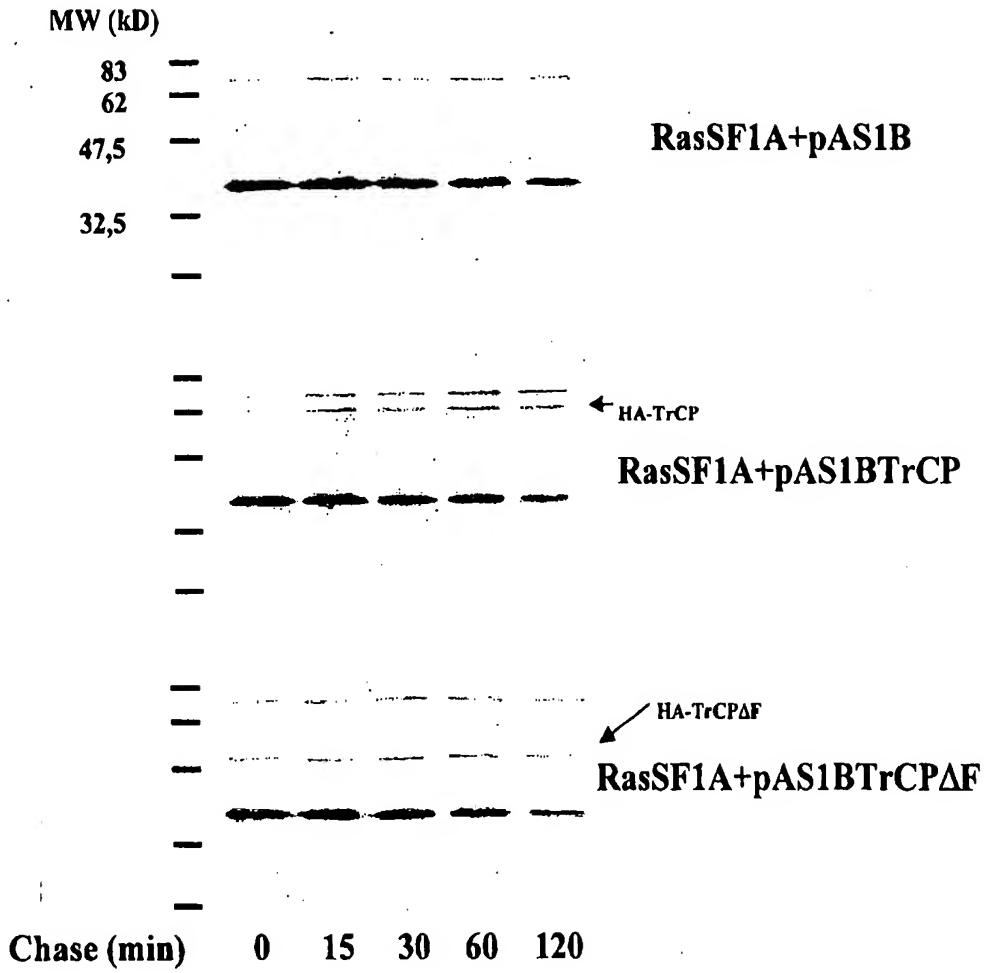

MW (kD)
83
62
47,5
32,5
RasSF1A+pAS1B
HA-TrCP
RasSF1A+pAS1BTrCP
HA-TrCPΔF
RasSF1A+pAS1BTrCPΔF
Chase (min) 0 15 30 60 120

Figure 18

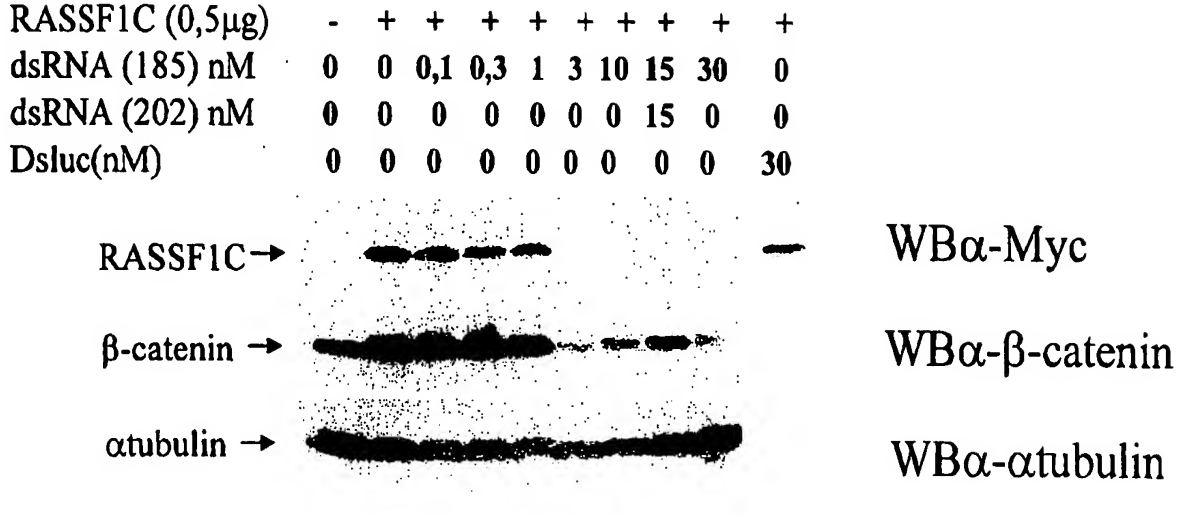

RASSF1C (0,5µg) - + + + + + + + + +
dsRNA (185) nM 0 0 0,1 0,3 1 3 10 15 30 0
dsRNA (202) nM 0 0 0 0 0 0 0 15 0 0
Dsluc(nM) 0 0 0 0 0 0 0 0 0 30
RASSF1C
β-catenin
αtubulin
WBα-Myc
WBα-β-catenin
WBα-αtubulin

Figure 19

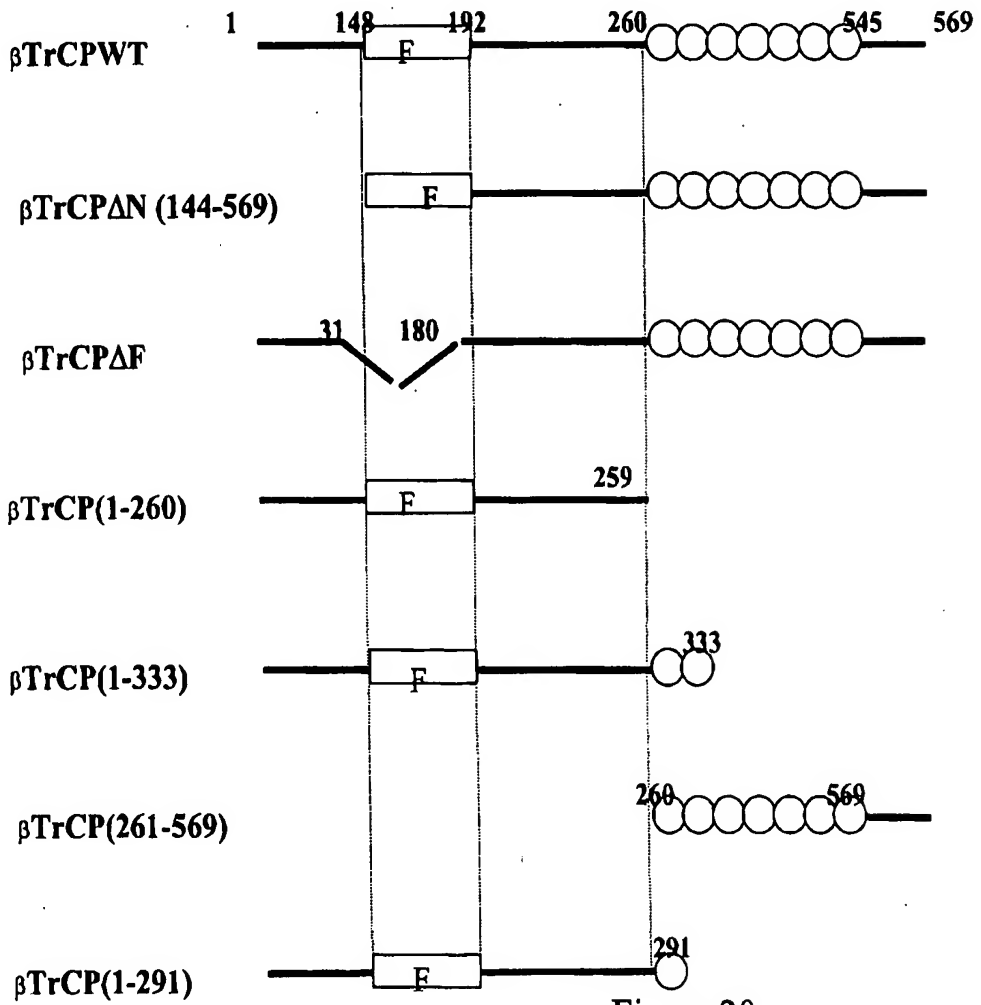

Interaction
RasSF1
βTrCPWT
1
148
192
260
545
569
F
+
βTrCPΔN (144-569)
F
+
βTrCPΔF
31
180
+
βTrCP(1-260)
259
F
-
βTrCP(1-333)
333
F
+
βTrCP(261-569)
260
569
-
βTrCP(1-291)
291
F
+

Figure 20